Wide-Field Microscopy using Microcamera Arrays

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ABSTRACT

A microcamera is a relay lens paired with image sensors. Microcameras are grouped into arrays to relay overlapping views of a single large surface to the sensors to form a continuous synthetic image. The imaged surface may be curved or irregular as each camera may independently be dynamically focused to a different depth. Microcamera arrays are akin to microprocessors in supercomputers in that both join individual processors by an optoelectronic routing fabric to increase capacity and performance. A microcamera may image ten or more megapixels and grouped into an array of several hundred, as has already been demonstrated by the DARPA AWARE Wide-Field program with multiscale gigapixel photography. We adapt gigapixel microcamera array architectures to wide-field microscopy of irregularly shaped surfaces to greatly increase area imaging over 1000 square millimeters at resolutions of 3 microns or better in a single snapshot. The system includes a novel relay design, a sensor electronics package, and a FPGA-based networking fabric. Biomedical applications of this include screening for skin lesions, wide-field and resolution-agile microsurgical imaging, and microscopic cytometry of millions of cells performed in situ.

Keywords: Wide-field microscopy, camera array, image processing, agile resolution

1. INTRODUCTION

Microscopy has usually been concerned with the imaging of small objects at high resolutions. Imaging large objects at high resolutions has typically required scanning a small aperture over a wide area, a strategy that can not be used to take a snapshot of a large object at an instant. For the DARPA AWARE Wide-Field project, we have developed cameras\textsuperscript{1} that are capable of imaging billions of pixels in a snapshot. Construction of this camera has included creating an architecture for coordinating hundreds of CMOS sensors to achieve simultaneous acquisition, stitching the images together, and calibration of the sensor fields. The use of such an architecture need not be confined to terrestrial use. By adapting the optics and electronic hardware developed for this camera, we can take snapshots of large areas at microscopic resolution. Such a capability likely has great uses in biomedical applications such as scanning for skin lesions, photographic cytometry, and microsurgical guidance. This is a natural extension of the close-up imaging\textsuperscript{2,3} principle, in which arrays of lenses are use to relay parts of a field to one or many detectors.

The DARPA AWARE Wide-Field camera is based on multiscale architecture.\textsuperscript{4} The architecture is illustrated in part (a) of Fig. 1. Rather than requiring a single set of optics to correct a wide field-of-view, which becomes prohibitively difficult to build as the resolution and field of view increases, one can divide the task of imaging parts of the field among many microcameras. A single objective, denoted by a brown ball, forms an intermediate image on a spherical surface inside the gray dome. The objective is monocentric so that all of the surfaces of the lens are spherical with the same center of curvature. The image formed by the objective is aberrated, however, the aberrations produced by the lens are invariant with field angle. The microcamera must correct these aberrations to produce a resolved image, but because of this invariance, the same microcamera can be used at all field angles.

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Figure 1. Part (a) An exploded view of the AWARE Wide-Field two gigapixel camera. A monocentric objective forms an image on an intermediate surface. Portions of this surface are relayed by microcameras to their respective sensors. Part (b) The prototype of the wide-field microscope based on the multiscale architecture developed for DARPA AWARE Wide-Field.
In the course of developing the DARPA AWARE Wide-Field camera,\textsuperscript{5–7} it was necessary to synchronize the acquisition of hundreds of the microcameras to capture a complete gigapixel image. It was conceived that instead of using the microcameras with an objective as in a multiscale camera, it is possible to use an array of microcameras to image a large area of an object at microscopic resolution. While the microcamera was designed to work with the objective, it can achieve satisfactory performance without it. The aberrations of the objective used in the DARPA AWARE 2 camera are fairly moderate, and so the aberrations required to be corrected by the microcamera are also moderate as well. Therefore the microcamera is able to be used as an imaging lens itself. In fact, the object-space numerical aperture of the microcamera is 0.11, comparable to many low-power 10× microscope objectives. Part (b) of fig. 1 shows microcameras arranged in an array looking at a surface. These are arranged in the same pattern that they would be in the multiscale camera looking at the surface that the objective would produce. However, the sample surface takes the place of the objective intermediate image surface. An advantage of microcamera arrays over monolithic lenses is that they may also be arranged to be conformal to irregular surfaces as well, and that because each microcamera has an individual focus, this surface may be varied dynamically to a limited extent.

Custom electronics were required to acquire and synchronize the acquisition of multiple microcameras. The sensor used in the microcamera was a 14 megapixel Aptina MT9F002 CMOS monochrome sensor array. The dimensions of this sensor were 4384 × 3288 with 1.4 μm pixels. A Multi-scale Camera Control Module (MCCM) was designed by Distant Focus Corporation (Champaign, IL), which included an Altera Cyclone IV 150K FPGA to control two CMOS sensors. The CMOS sensors transmitted their data through the Aptina HiSPi (High-Speed Serial Pixel Interface) to the FPGA to be buffered in 512 MB DDR RAM. The buffer memory saves the acquired frames until the frames can be retrieved. The module communicates through a 1000B-T Ethernet physical link layer via TCP/IP and UDP/IP protocols to a Linux PC, which issues control instructions and receives frames from all cameras through this interface. All of the MCCM devices were connected to a gigabit Ethernet switch. Also included was a HDMI (High-Definition Multimedia Interface) port that displayed a live output of the CMOS sensors for debugging and a USB 2.0 port as an alternate means of camera control and image capture.

The microcamera for the AWARE 2 project consists of an optical barrel assembly, the CMOS sensor which can be translated for focus on a lead screw, and a servo motor to drive the focus. These parts are shown in Fig. 2. The optical barrel relay lens has a magnification of 0.49, with a 10 mm × 10 mm field of view relayed to a sensor area of 4.9 mm × 4.9 mm. A distortion of about −3% is present. There are four lenses in the microcamera. Two are made from the crown plastic Zeonex E48R (Zeonex, Louisville, KY, $n_d = 1.531160, V_d = 56.043828$). The other two are made from the “flint” plastic polycarbonate ($n_d = 1.585470, V_d = 29.909185$). Unfortunately, the actual design performance was significantly degraded by residual stress birefringence when molding the polycarbonate, and therefore alternatives to this plastic should be considered for future designs. The image-space $f/#$ of the camera is 2.17, which helps to concentrate the available light and may be particularly helpful for imaging fluorescence. A small $f/#$ is required to image at Nyquist-limited performance on a sensor with 1.4 μm pitch pixels. The sensor was attached to a flexible circuit board that also served as the signal cable to the sensor controller electronics. The flexible cable was also required as the sensor was translated for focus on a leadscrew, so rigid mounting of the sensor chip could not be used. The lead screw was turned by a servo motor to translate the sensor through 0.070 mm of travel. Two bushings to the side of the CMOS sensor fitted into two pins on the optical barrel to precision align the barrel to the optical axis. The completed microcamera module is shown at the bottom of the figure.

The nominal performance of the microcamera without the objective is shown in Fig. 3. At the top of the figure is a ray trace of the object points through to the image sensor plane. The nominal modulation transfer function curve is shown at the bottom of the figure. Even without the objective, good performance can be achieved up to the Nyquist frequency except at the very edge of the field. Despite the fact that the microcameras were not specifically designed for this purpose, they still provide acceptable performance. Future microcamera arrays specifically designed for microscopy can be expected to improve performance over the current design for the DARPA AWARE Wide-Field camera.
Figure 2. The parts of the plastic optics microcamera including the barrel, the sensor module, and the assembled micro-camera. A servo motor behind the microcamera provides focus capability that is attached with a clip wire onto the sensor module.

Also, for a comparison, we also constructed a microcamera using spherical glass elements rather than aspheric molded plastic elements. A picture of this microcamera and its prescription is shown in Fig. 4. Because of the aforementioned birefringence problems with plastic, it was desired to understand the performance improvements that might be obtained with less birefringence present. Properly made and polished glass elements typically have little or no birefringence and therefore make a good benchmark to compare with. Different plastic choices will likely greatly diminish the problems encountered with birefringence and therefore make this issue less problematic in the future. The glass microcamera is f/3 with a magnification of 0.37 so that the nominal resolution of the glass microcamera is not as high as the plastic microcamera.

To explore the use of the AWARE Wide-Field architecture for wide-field microscopy, we first used the glass element microcamera to image a zebrafish embryo model. This model has exceptional transparency in the embryo stage to allow deep imaging into the animal. The zebrasishes were in a polystyrene transparent petri dish. Instead of using bright-field illumination, a dark-field like setup was used where a black background was placed under the petri dish and the sample as illuminated by a fiber-optic light from the side. The scattering from surfaces in the sample produced the contrast in a manner similar to dark-field microscopy. The results of this imaging setup are demonstrated in Figure 5, showing the entire 12 mm imaged distance at the object plane, and an inset with details on a few embryos at the center. The diffraction limited performance is maintained with 0.06 object space numerical aperture over the object plane.

Using the plastic element microcameras, two demonstrations were performed of simultaneous capture of seven microcameras. The first, shown in part (a) of Fig 6, is seven microcamera images of a 1951 USAF target stitched into a single image. A second demonstration is the simultaneous capture of the petri dish of zebrafish embryos.
Figure 3. The ray trace of the plastic optics microcamera and the modulation transfer function without the objective. The aberration of the AWARE 2 design is modest, so that even without the objective, the microcamera has reasonable imaging performance.

Figure 4. The microcamera built from spherical glass optical elements. This is designed to be interchangeable with the plastic barrel microcamera.
Figure 5. A 14-megapixel microscopic image of Zebrafish embryos taken with the glass element microcamera shown in Fig. 4.

Figure 6 shows the images composed from the seven microcamera images including white-boxed insets showing detail of particular regions. While the plastic lens performance currently does not match or exceed the resolution of the glass barrel optics, we believe that with better choices of plastic materials these defects can be corrected and superior performance to the glass barrel optics can be achieved.

We have demonstrated the simultaneous capture from multiple microcameras, each acting as both a microscope objective and a relay lens. This system enables extended objects to be imaged without a scanned aperture at a microscopic resolution. As the technology of aspheric molded optic reproduction continues to improve, such an approach may eventually become economically attractive as a replacement for many conventional microscopic systems, especially ones that require high throughput. As the AWARE Wide-Field camera is adopting newer plastics with higher homogeneity and lower birefringence, we expect that these systems can achieve a performance more competitive to precision commercial microscopy systems.

Acknowledgments
This project was supported by the DARPA MTO AWARE program under contract HR-0011-10-C-0073. We thank Prof. David Tobin of the Department of Molecular Genetics and Microbiology at Duke University for his assistance with the zebrafish embryo animal model.

REFERENCES
Figure 6. Part (a) is a simultaneous capture from seven microcameras of a 1951 USAF target. Part (b) is a simultaneous capture of a petri dish of zebrafish embryos. For comparison, a 14-megapixel microscopic image of Zebrafish embryos taken with the glass element microcamera shown in Fig. 4.